p63 EXPRESSION IN THE SALIVARY GLAND NEOPLASMS ADENOID CYSTIC CARCINOMA, POLYMORPHOUS LOW-GRADE ADENOCARCINOMA AND MONOMORPHIC ADENOMA

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ABSTRACT

Objectives:

The purpose of this study was to compare the extent of p63 immunoreactivity in the malignant salivary gland neoplasms adenoid cystic carcinoma (ACC) and polymorphous low-grade adenocarcinoma (PLGA) and to compare it to the expression of this marker in the benign salivary gland tumor monomorphic adenoma (including canalicular adenoma and basal cell adenoma).

p63, a selective histochemical marker of basal/stem cells of stratified epithelium and of myoepithelial cells, is a p53 homologue which plays an essential role in both morphogenesis of the epidermis and limb development. P63 immunoreactivity has been demonstrated in squamous cell and urothelial carcinomas, whereas it is generally absent in most non-squamous cell carcinomas.

Little is known about the expression of p63 in head and neck salivary gland tumors have been published to date.

Study Design:

Formalin-fixed paraffin-embedded sections from 49 salivary gland neoplasms (17 monomorphic adenomas, 17 PLGA and 15 ACC) accessioned between 1989 and 2002 by the Department of Pathology, Long Island Jewish Medical Center, New Hyde Park, NY, were stained with an anti-p63 monoclonal antibody.

Results:

Nuclear p63 reactivity was uniformly positive in PLGA (17/17, 100%).

Positive reactivity was also identified in the majority of ACCs (13/15, 87%), primarily in the non-luminal myoepithelial-like cells surrounding the true luminal cells.

The canalicular adenomas did not exhibit any p63 immunoreactivity. All of the basal cell adenomas of parotid origin stained strongly for p63, with staining localized to the peripheral tumor cells situated adjacent to the connective tissue stroma. None of the basal cell adenomas from the upper lip stained with p63.

In the normal surrounding salivary gland tissue, p63 reactivity was identified only focally in the basal cells of striated ducts.

Conclusions:

We found that p63 is strongly expressed in basal cell adenomas of parotid origin, ACC and PLGA. Canalicular adenomas did not demonstrate any p63 staining, consistent with this tumor's putative ductal luminal cell differentiation.

Our results suggest that the neoplastic cells in PLGA may represent either myoepithelial cells or a population of p63-positive epithelial stem/reserve cells similar to the basal cells of stratified epithelium.

P63 does not appear to be an ideal marker for distinguishing between ACC and PLGA in equivocal cases, or benign from malignant salivary gland neoplasms.

INTRODUCTION

P63, a selective histochemical marker of basal/stem cells of stratified epithelium and of myoepithelial cells[1], is a p53 homologue which plays an essential role in both morphogenesis of the epidermis and limb development[2]. Recently[3], it has been shown that p63 is required for p53-dependent apoptosis in response to DNA damage.

Structurally, the p63 gene is located on chromosome 3q27-29[4] and encodes three isoforms, p63α, p63β and p63γ, which share a common amino-terminal end but differ at their carboxyl-terminal ends due to alternate mRNA splicings[5]. In addition, the gene for p63 contains a second promoter located upstream of exon 3, which gives rise to three amino-terminal deleted (p63ΔN) transcripts. The deleted amino-terminal region codes for a domain that is capable of trans-activating p53 target genes, including those involved in apoptosis. Consequently, p63ΔN isoforms lack p53-like activity. However, because they maintain their ability to compete with p53 for DNA target sites, p63ΔN isoforms appear to negatively regulate p53 activity[6]. These isoforms are the predominant type found in epithelial stem cells.

The key role of p63 in embryogenesis has been demonstrated by knock out experiments in mice[7]. p63-deficient mice exhibit truncated forelimbs, absent hind limbs, absence of teeth, hair follicles and mammary glands. In p63-deficient mice, the oral epithelium consists of only a single layer of flattened epithelial cells[7]. It appears that these deficiencies are caused by a perturbation of ectodermal-mesenchymal signaling,

likely due to a lack of stem cells necessary to sustain epithelial morphogenesis and renewal[8]. In the absence of p63 expression, the basal cells undergo terminal differentiation, effectively running out of stem cells. It has been hypothesized that the preferential expression of the transactivation-deficient p63ΔN isoforms protects the basal epithelial cells from p53-induced apoptosis[6].

In humans, p63 gene mutations are involved in the pathogenesis of at least five autosomal dominantly inherited syndromes characterized by various combinations of limb deformities[9,10]. The most common of these is ectrodactily, ectodermal dysplasia and facial clefts syndrome (EEC), which closely resembles the phenotype seen in p63-deficient homozygous mice[5].

At the cellular level, p63 is constitutively expressed in the basal cell nuclei of many normal stratified epithelial tissues[6], including skin, squamous epithelium of the oral cavity, esophagus and cervix, prostatic acinar basal cells, esophageal mucosal gland ducts, and urothelium. P63 is strongly expressed in breast myoepithelial cells[11] and is also expressed in normal thymus and in thymomas[13]. P63 expression is not found in the basal non-stratified epithelium of the stomach, colon or small intestine[12], nor is it detected in normal mesenchymal or neural/neuroendocrine tissue[13].

Myofibroblasts are consistently negative for p63. This is in contrast to other myoepithelial markers such as calponin and smooth muscle actin, which can show variable myofibroblastic staining.

Barbareschi et al[11] identified a small subset of p63 positive, alpha-smooth muscle actin negative basal cells in the human breast, which may represent the breast somatic stem cell.

A direct role for p63 in tumorogenesis has not been demonstrated to date, although amplification of the 3q27 region has been seen in a number of tumors, including squamous cell carcinoma[4]. This is suggestive of a putative role as an oncogene rather than as a tumor suppressor gene[6].

In neoplastic tissue, p63 immunoreactivity has been demonstrated in squamous cell and urothelial carcinomas[14], whereas it is generally absent in most non-squamous cell carcinomas[11]. Adenocarcinomas reportedly do not express p63[13].

This loss of immunoreactivity in non-squamous cell malignant neoplasms has been exploited to aid in identifying microinvasion in cases of DCIS and LCIS[11], and to differentiate benign prostatic tissue from adenocarcinoma[14-15]. In the case of poorly differentiated metastatic carcinomas, positive immunostaining for both p63 and CK5/6 is highly predictive of a primary tumor of squamous epithelial origin[14].

To date, no in depth studies on the expression of p63 in malignant head and neck salivary gland tumors have been published. Di Como et al[13] reported positive p63 reactivity in 4/4 benign mixed tumors of the parotid gland and 2/4 unspecified salivary gland carcinomas. In abstract form, Krane et al[16] reported p63 positivity in a limited number of salivary gland neoplasms, including ACC, PLGA and basal cell adenoma.

The purpose of this study was to compare the extent of p63 immunoreactivity in adenoid cystic carcinoma, polymorphous low-grade adenocarcinoma and monomorphic adenoma.

MATERIALS AND METHODS

Formalin-fixed paraffin-embedded sections from 49 salivary gland neoplasms (17 monomorphic adenomas, 17 PLGA and 15 ACC) accessioned between 1989 and 2002 were retrieved from the files of the Department of Pathology, Long Island Jewish Medical Center.

The H&E stained slides were independently reviewed by two experienced pathologists (RK, TB) and agreement was reached on all cases included in this study using accepted criteria[17].

Microwave heat-induced epitope retrieval (750 W microwave at medium power, 30 minutes) was performed using the Trilogy™ system (Cell Marque, Hot Springs AK) as per the manufacturer's directions and then treated with anti-human p63 monoclonal antibody that detects all isoforms (Ab-4, clones 4A4 and Y4A3; Neomarkers, Fremont CA) at a 1:50 dilution.

The antibody-antigen complex was detected using an indirect biotin-avidin system as per the manufacturer's instructions (Ventana Basic DAB Detection Kit™, Ventana Medical Systems, Tucson AZ).

Fetal adrenal cortex was used as positive control. Normal salivary gland tissue was used as the negative control. When present, stratified squamous epithelium was used as an internal positive control.

Immunoreactivity was independently assessed by all study participants. Agreement was reached on all 49 specimens examined. Immunoreactivity was considered positive if greater than 10% of the tumor cells stained and was graded as weak (10-25%), mild (26-50%), moderate (51-75%) and strong (76-100%).

RESULTS

The ACC group, comprising 12 women and 3 men, had a median age of 65 years (range: 37-89 years). The tumor group included 8 lesions of minor salivary gland origin (4 palate, 3 soft tissue of the neck, 1 maxillary sinus) and 7 lesions of major gland origin (5 parotid, 2 submandibular gland). The histological subtypes include 4 cribriform, 3 tubular and 8 solid patterns.

The median age of the PLGA group was 67 years (range: 38-73 years) and consisted of 13 female patients and 4 male patients. All tumors arose from minor salivary gland tissue, primarily palate (8) and buccal mucosa (4), except for one of parotid origin.

The monomorphic adenoma group, comprising 14 women and 3 men, had a median age of 66 years (range: 41-97). The tumors were located in the upper lip (9), parotid (4), buccal mucosa (3), and hard palate (1) and comprised 6 canalicular adenomas and 11 basal cell adenomas.

P63 nuclear staining (Table 1) was uniformly positive in all PLGAs (17/17, 100%), with at least 25% of the tumor cells being positive (Figure 1).

Positive reactivity was also identified in the majority of ACCs (13/15, 87%; Figure 2). Staining was located primarily in the non-luminal myoepithelial-like cells surrounding the true luminal cells. The small polygonal luminal cells characterized by pale eosiniphilic cytoplasm failed to stain for p63. The two cases of ACC with no immunoreactivity and the single case with weak p63 immunoreactivity were predominantly of the solid variant. However, 5 of 8 solid ACCs stained strongly.

The monomorphic adenomas variably expressed the p63 antigen (6/17, 35%). Subdividing the monomorphic adenomas (Table 2) demonstrated no staining in the canalicular adeoma subtype (Figure 3a) and variable staining in the basal cell adenomas (Figure 3b). All basal cell adenomas of parotid origin stained strongly positive for p63. Staining was localized to the peripheral tumor cells located adjacent to the connective tissue stroma. None of the basal cell adenomas from the upper lip stained for p63.

In the normal surrounding salivary gland tissue, immunoreactivity was focally detected in the basal cells of striated ducts.

	ADENOID CYSTIC CARCINOMA	POLYMORPHOUS LOW GRADE ADENOCARCINOMA	MONOMORPHIC ADENOMA
Total number of samples	15	17	17
Negative staining	2 (Note 1)	0	11
Weak staining (10-25% of cells)	1 (Note 2)	0	0
Mild staining (26-50%)	0	4	1
Moderate staining (51-75%)	1	3	1
Strong staining (76-100%)	11	10	4
% samples with positive staining	**87%**	**100%**	**35%**

Table 1: Summary of p63 staining results

Note 1: Both lesions with no staining were solid variants of ACC
Note 2: This lesion was mostly solid variant of ACC

	Canalicular Adenomas (N=6)			**Basal Cell Adenomas (N=11)**		
Origin	Parotid	Upper Lip	Other Minor Gland	Parotid	Upper Lip	Other Minor Gland
Negative staining	-	5	1	-	4	1
Weak staining (10-25% of cells)	-	-	-	-	-	-
Mild staining (26-50%)	-	-	-	-	-	1[1]
Moderate staining (51-75%)	-	-	-	-	-	1
Strong staining (76-100%)	-	-	-	4	-	-
% samples with positive staining	**0%**			**66%**		

Table 2: Summary of p63 staining results by subtype of monomorphic adenoma (canalicular adenoma versus basal cell adenoma) and by site of origin

Note 1: This lesion had features of both canalicular adenoma and basal cell adenoma. The basal cell adenoma area stained for p63, whereas the canalicular adenoma area did not.

DISCUSSION

The participation of myoepithelial cells in certain salivary gland neoplasms such as adenoid cystic carcinoma is generally accepted[17]. Our results are in agreement with these findings. We found strong positive p63 nuclear staining in 12 of 15 ACCs examined. The two cases of ACC with no immunoreactivity and the single case with weak p63 immunoreactivity were predominantly of the solid variant. Nevertheless, most solid ACCs examined showed strong p63 immunoreactivity,.

Polymorphous low-grade adenocarcinoma appears to show little evidence of myoepithelial differentiation, although this view has been challenged[18]. While Jones et al[18] reported focal alpha-smooth muscle actin in 5 of 6 PLGAs examined, Regezi et al[19] reported positive immunstaining for S100 and vimentin but only weak staining for muscle-specific actin in 2 of 15 PLGAs. Prasad et al[20] compared the immunoreactivity of 13 cases of ACC, 26 cases of PLGA and 17 cases of canalicular adenomas stained with the smooth muscle-specific monoclonal antibodies α-smooth muscle actin, smooth muscle myosin heavy chain and calponin and demonstrated that the ACCs consistently stained positive for myoepithelial markers in ACCs and lacked staining in PLGAs and canalicular adenomas. They concluded that there was no evidence of myoepithelial differentiation in PLGA. The results of Araujo et al[21], in which only 3 of 28 PLGAs demonstrated mild muscle specific actin immunoreactivity, appear to confirm this interpretation. No myoepithelial-like cells were identified in five cases examined by transmission electron microscopy[21].

Our results suggest that the neoplastic cells in PLGA may represent either myoepithelial cells or a population of p63-positive epithelial stem/reserve cells similar to the basal cells of stratified epithelium. In light of previously published immunohistochemical and ultrastructural studies suggesting the absence of myoepithelal participation in the histogenesis of PLGA, our findings could be interpreted as suggesting that the neoplastic cells in PLGA may represent a population of p63-positive epithelial stem/reserve cells similar to the basal cells of stratified epithelium. However, in the absence of double-staining experiments on the same specimens using both p63 and cytoplasmic markers of myoepithelial cell origin (e.g. calponin and smooth muscle actin), the involvement of myoepithelial cells in the histogenesis of PLGA cannot be ruled out.

We were unable to demonstrate any p63 staining in the canalicular adenomas. This is in agreement with previous findings that have demonstrated an absence of myopeithelial differentiation in canalicular adenomas both by immunohistochemical[20] and ultrastructural studies[22], and is consistent with this tumor's putative ductal luminal cell differentiation[23].

Variable p63 staining was identified in the basal cell adenomas. All of the basal cell adenomas of parotid origin stained strongly for p63, with staining localized to the peripheral tumor cells situated adjacent to the connective tissue stroma. Zarbo et al[23] reported similar staining patterns using the myoepithelial markers troponin, smooth muscle myosin heavy chain and α-smooth muscle actin. None of the basal cell adenomas from the upper lip in our study stained for p63. This is not incompatible with the ultrastructural and immunohistochemical studies that have demonstrated relative

differences in the extent of ductal luminal, myoepithelial and basal cell differentiation between different basal cell adenomas[17], which may in part be a function of the site of origin of the neoplastic tissue.

CONCLUSIONS

Nuclear p63 reactivity was uniformly positive in PLGA. Since p63 is a selective histochemical marker of both basal/stem cells of stratified epithelium and of myoepithelial cells, our results suggest that the neoplastic cells in PLGA may represent either myoepithelial cells or a population of p63-positive epithelial stem/reserve cells similar to the basal cells of stratified epithelium.

Positive p63 reactivity was also identified in the majority of ACCs.

Monomorphic adenomas variably expressed the p63 antigen, with the canalicular adenomas demonstrating no staining and the basal cell adenomas of parotid gland origin exhibiting strong staining. In the normal surrounding salivary gland tissue, p63 reactivity was identified only focally in basal cells of striated ducts.

In summary, we find that p63 does not appear to be an ideal marker for distinguishing between ACC and PLGA in equivocal cases, or benign from malignant salivary gland neoplasms.

Figure 1: Polymorphous low-grade adenocarcinoma adjacent to overlying normal surface epithelium. (A) Hematoxylin and eosin stain, and (B) p63 demonstrating strong nuclear staining in the neoplastic cells. Note the positive p63 staining in the basal layer of normal stratified squamous epithelium (original magnification 100x).

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Figure 2: Adenoid cystic carcinoma. (A) Hematoxylin and eosin stain, and (B) p63. Note that the polygonal luminal cells (arrow) do not stain, whereas the surrounding non-luminal myoepithelial/basal reserve cells show strong nuclear p63 immunoreactivity (original magnification 200x).

Figure 3a: Monomorphic adenoma, canalicular adenoma subtype. (A) Hematoxylin and eosin stain, and (B) p63. Note the positive p63 staining in the normal basal epithelium and the lack of nuclear staining in the neoplastic cells (original magnification 100x)

Figure 3b: Monomorphic adenoma, tubulo-trabecular basal cell adenoma subtype. (A) Hematoxylin and eosin stain, and (B) p63 demonstrating nuclear staining in the neoplastic cells. Note that the p63 staining is primarily localized to the peripheral tumor cells adjacent to the connective tissue stroma (original magnification 100x).

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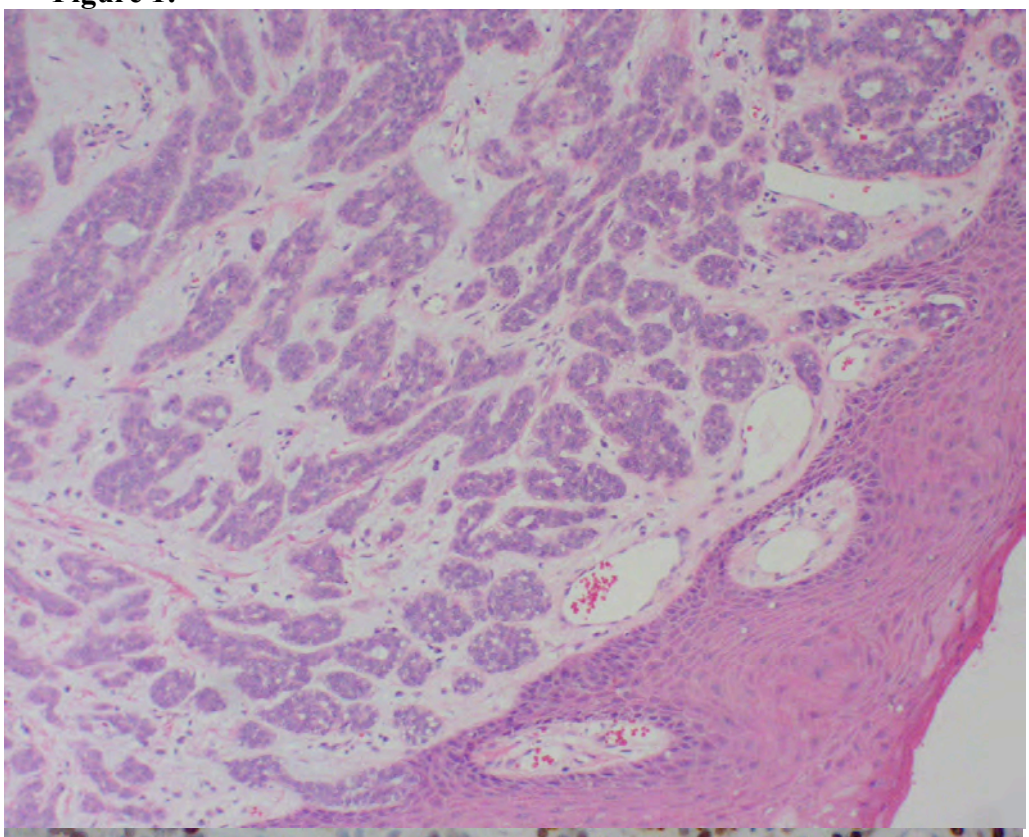
Figure 1:

A



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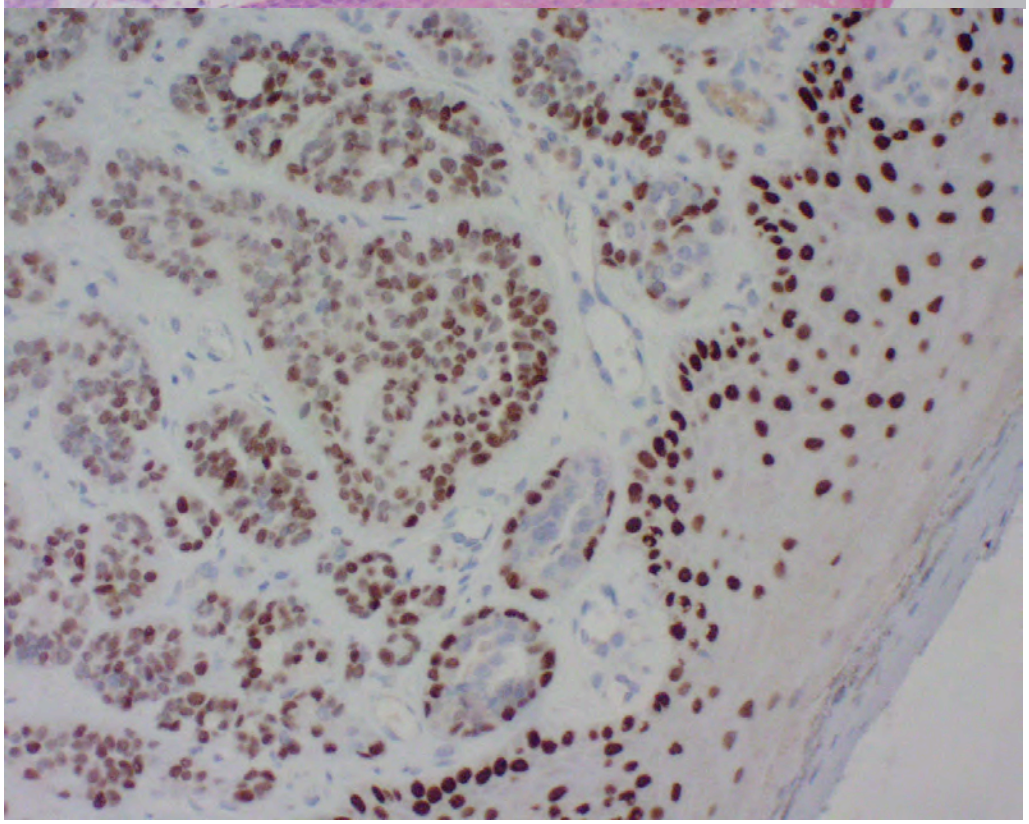
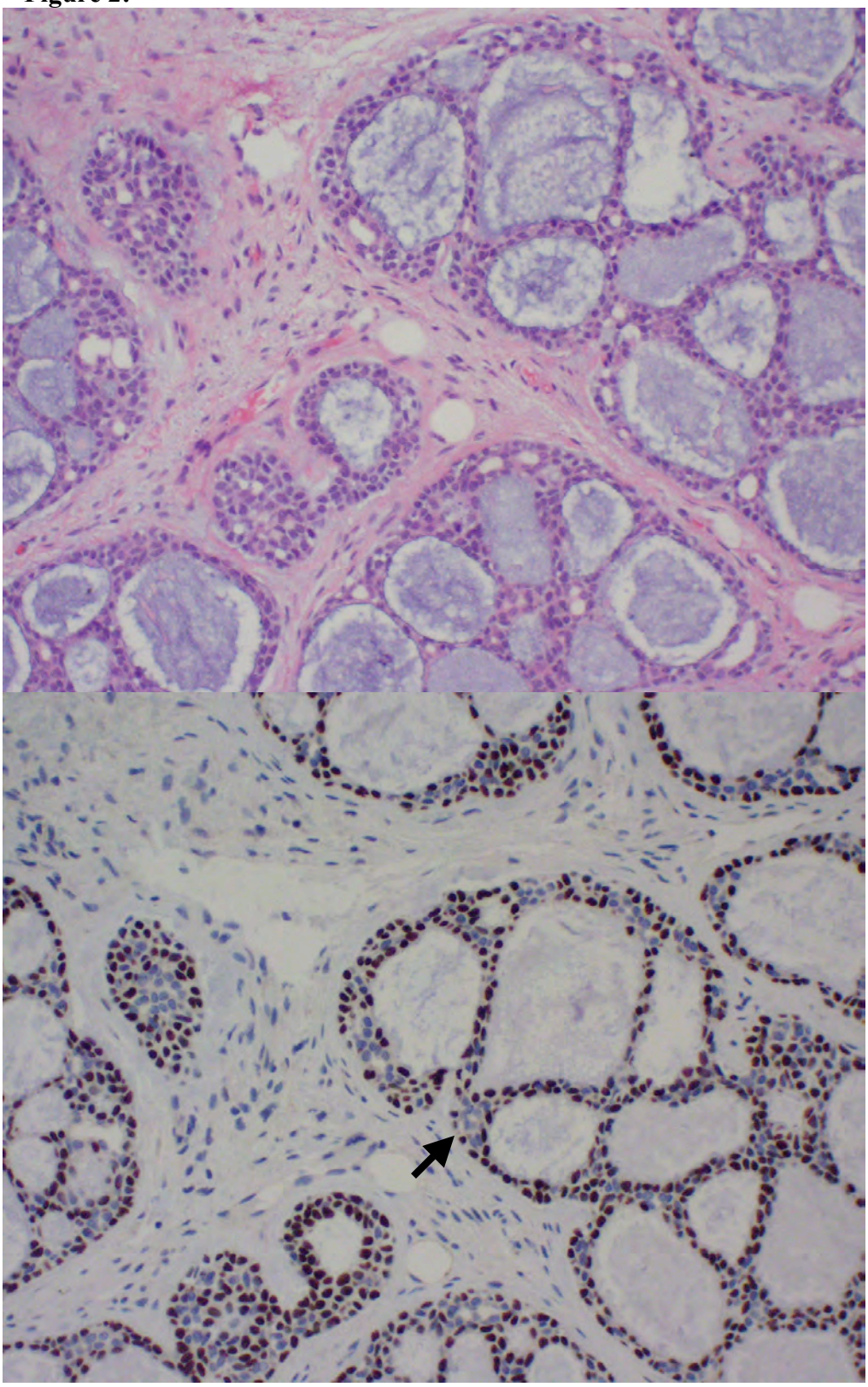


Figure 2:



A

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Figure 3a:

B

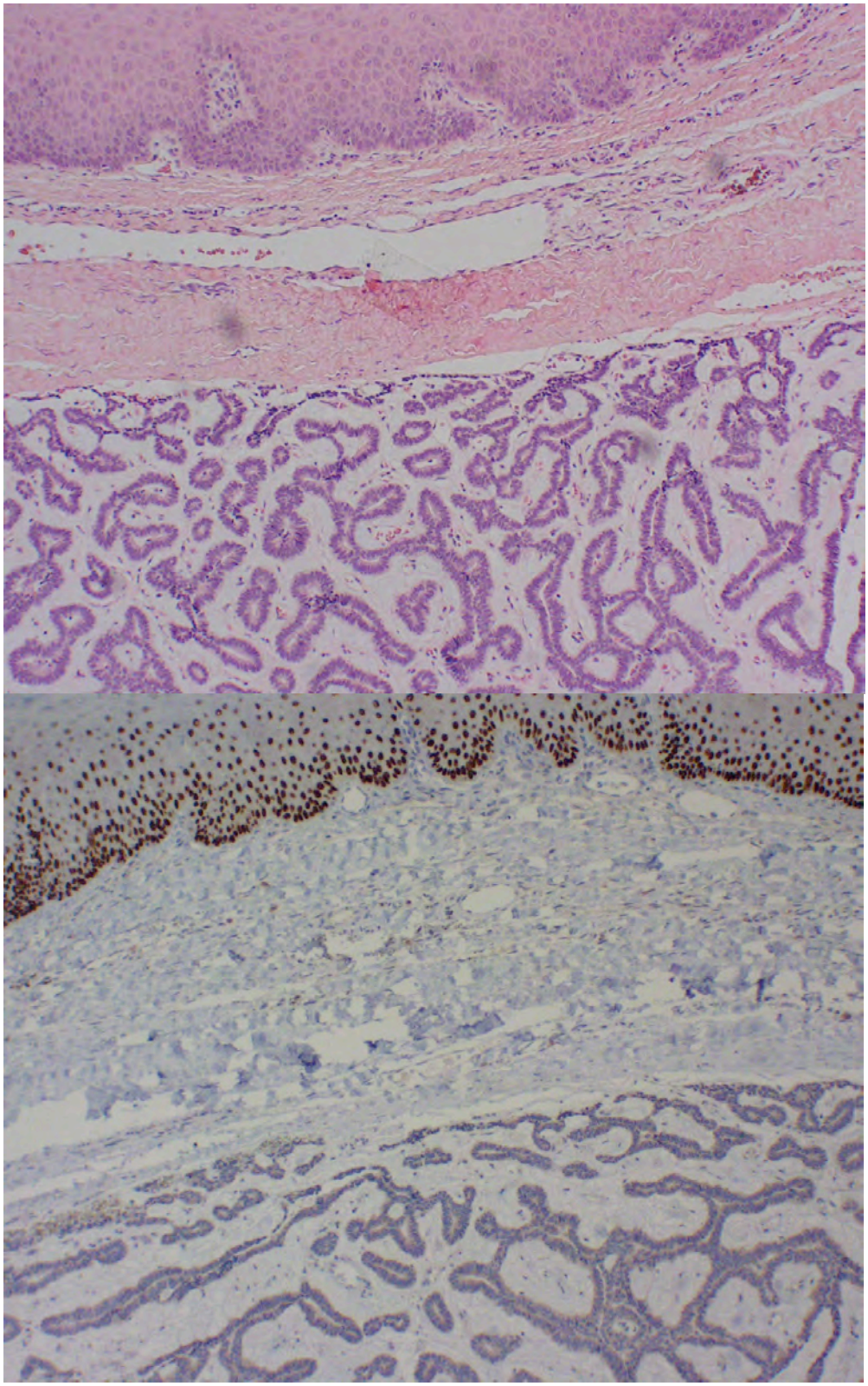
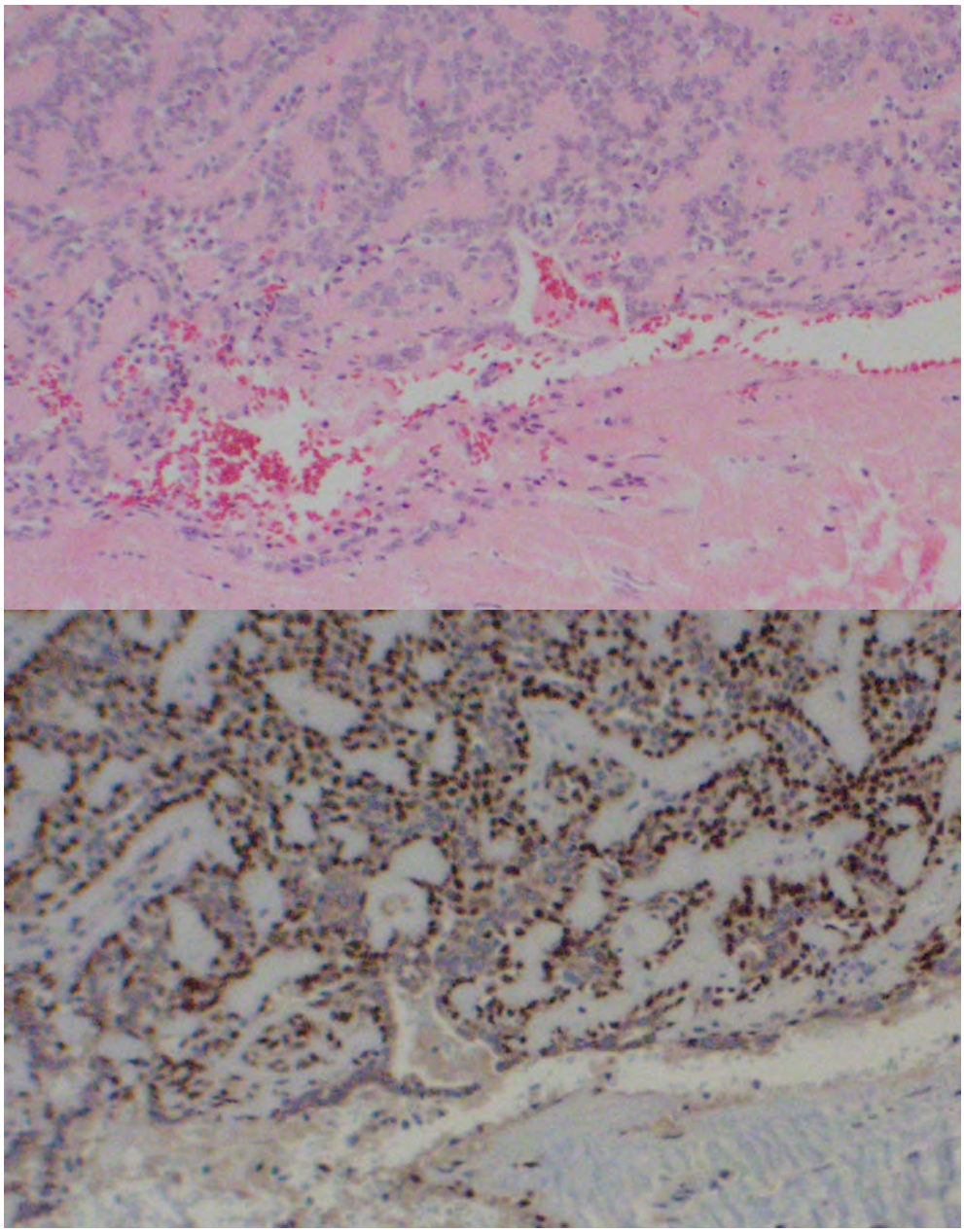


Figure 3b:

A



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REFERENCES

1 Reis-Filho JS, Schmitt FC. Taking advantage of basic research: p63 is a reliable myoepithelial and stem cell marker. Adv Anat Pathol 2002; 9: 280-9.

2 Yang A. Schweitzer R, Sun D, Kaghad M, Walker N, Bronson RT, et al. P63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. Nature 1999; 398: 714-8.

3 Flores ER, Tsai KY, Crowley D, Sengupta S, Yang A, McKeon F, et al. p63 and p73 are required for p53-dependent apoptosis in response to DNA damage. Nature 2002; 416: 560-4.

4 Nylander K, Dabelsteen E, Hall PA. The p53 molecule and its prognostic role in squamous cell carcinomas of the head and neck. J Oral Pathol Med 2000; 29: 413-25.

5 Strano S, Rossi M, Fontemaggi G, Munarriz E, Soddu S, Sacchi A, et al. From p63 to p53 across p73. FEBS Letts 2001; 490: 163-70.

6 Yang A, Kaghad M, Caput D, McKeon F. On the shoulders of giants: p63, p73 and the rise of p53. Trends Genet 2002; 18: 90-5.

7 Mills AA, Zheng B, Wang XJ, Vogel H, Roop DR, Bradley A. P63 is a p53 homologue required for limb and epidermal morphogenesis. Nature 1999; 398: 708-13.

8 Pellegrini G, Dellambra E, Golisano O, Martinelli E, Fantozzi I, Bondanza S, et al. p63 identifies keratinocyte stem cells. PNAS 2001; 98: 3156-61.

9 Brunner HG, Hamel BC, van Bokhoven H. The p63 gene in EEC and other syndromes. J Med Gen 2002; 39: 377-81.

10 Brunner HG, Hamel BC, van Bokhoven H. P63 gene mutations and human developmental syndromes. Am J Med Gen 2002; 112: 284-90.

11 Barbareschi M, Pecciarini L, Cangi MG, Macri E, Rizzo A, Viale G, et al. P63, a p53 homologue is a selective nuclear marker of myoepithelial cells of the human breast. Am J Surg Pathol. 2001; 25: 1054-60.

12 Glickman JN, Yang A, Shahsafaei A, McKeon F, Odze RD. Expression of p53-related protein p63 in the gastrointestinal tract and in esophageal metaplastic and neoplastic disorders. Hum Pathol 2001; 32: 1157-65.

13 Di Como, CJ, Urist MJ, Babayan I, Drobnjak M, Hedvat CV, Teruya-Feldstein J, et al. p63 expression profiles in human normal and tumor tissues. Clin Cancer Res 2002; 8: 494-501.

14 Kaufmann O, Fietze E, Mengs J, Dietel M. Value of p63 and cytokeratin 5/6 as immunohistochemical markers for the differential diagnosis of poorly differentiated and undifferentiated carcinomas. Am J Clin Pathol 2001; 116: 823-30.

15 Parson JK, Gage WR, Nelson WG, De Marzo AM. P63 protein expression is rare in prostate adenocarcinoma: implications for cancer diagnosis and carcinogenesis. Urology 2001; 58: 619-24.

16 Krane JF, McKeon F, Faquin WC. Expression of p63, a p53 homologue, in normal and neoplastic salivary gland (Abstract 881). Mod Pathol 2001; 14: 151A

17 Ellis GL, Auclair PL. Tumors of the salivary gland. Atlas of tumor pathology. Third series. Fascicle 17. 1995. Armed Forces Institute of Pathology, Washington, DC.

18 Jones H, Moshtael F, Simpson RH. Immunoreactivity of α-smooth muscle actin in salivary gland tumors: a comparison with S-100 protein. J Clin Pathol 1994; 45: 938-40.

19 Regezi JA, Zarbo RJ, Stewart JC, Courtney RM. Polymorphous low-grade adenocarcinoma of minor salivary gland. A comparative histologic and immunohistochemical study. Oral Surg Oral Med Oral Pathol 1991; 71: 469-75.

20 Prasad AR, Savera AT, Gown AM, Zarbo RJ. The myoepithelial immunophenotype in 135 benign and malignant salivary gland tumors other than pleomorphic adenoma. Arch Pathol Lab Med 1999; 123: 801-6.

21 Araujo V, Sousa S, Jaeger M, Jaeger R, Loyola A, Crivelini M, et al. Characterization of the cellular component of polymorphous low-grade adenocarcinoma by immunohistochemistry and electron microscopy. Oral Oncol 1999; 35: 164-72.

22 Guccion JG, Redman RS. Canalicular adenoma of the buccal mucosa; an ultrastructural and histochemical study. Oral Surg Oral Med Oral Pathol 1986; 61: 173-8.

23 Zarbo RJ, Prasad AR, Regezi JA, Gown AM, Savera AT. Salivary gland basal cell and canalicular adenomas: Immunohistochemical demonstration of myoepithelial cell participation and morphogenetic considerations. Arch Pathol Lab Med 2000; 124: 401-5.